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Supplemental Data

Mutation in *NSUN2*, which Encodes an RNA Methyltransferase, Causes Autosomal-Recessive Intellectual Disability

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Figure S1. Photographic Images of Affected Individuals from the Pakistani Family

Slight elongation of the face and nose, and short philtrum are noticeable in the older girls (II:3 and II:4), with the nasal tip protruding below the alae. Right eye strabismus is apparent in individual II:3. Intercommissural distance seems somewhat long in all the individuals. A full clinical description can be requested from the corresponding author.

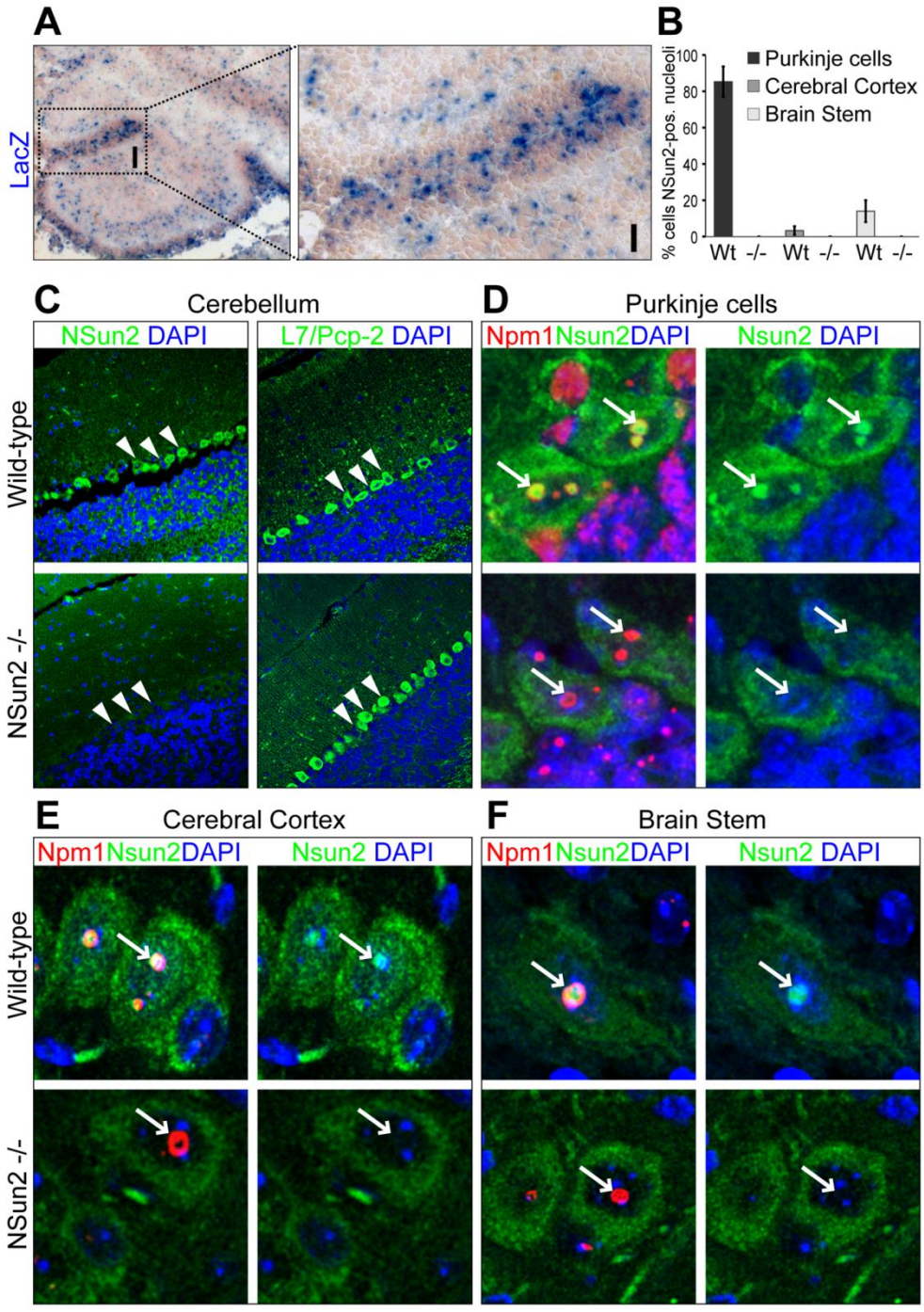
A
LacZ
B
% cells NSun2-pos. nucleoli
100
80
60
40
20
0
Purkinje cells
Cerebral Cortex
Brain Stem
Wt -/- Wt -/- Wt -/-
C
Cerebellum
NSun2 DAPI
L7/Pcp-2 DAPI
Wild-type
NSun2 -/-
D
Purkinje cells
Npm1Nsun2DAPI
Nsun2 DAPI
E
Cerebral Cortex
Npm1Nsun2DAPI
Nsun2 DAPI
F
Brain Stem
Npm1Nsun2DAPI
Nsun2 DAPI

Figure S2. Localization of Nsun2 in Brain Regions of Wild-Type and Knockout Mice

(A) Detection of β-Galactosidase (LacZ; blue) as reporter for location of NSUN2 in the cerebellum of *NSun2*+/- mice at postnatal day 3. Cells are counterstained with nuclear fast red.

(B) Quantification of cells staining positive for NSUN2 in nucleoli in Purkinje cells, cerebral cortex and brain stem in sections of wild-type (Wt) and *NSun2* -/- (-/-) mice at postnatal day 23. Error bars represent the standard deviation.

(C–F) Examples for nucleolar localization of NSUN2 (green) quantified in (B) in cerebellum (C), Purkinje cells (D), cerebral cortex (E) and brain stem (F) using brain sections of wild-type (C–F; upper panels) and *NSun2*-/- (C–F, lower panels) animals. Arrowheads in (C) mark Purkinje cells and arrows in (D–F) indicate nucleoli. Cells are counterstained with L7/Pcp-2 (green) to mark Purkinje cells (C), NPM1 (red) as a nucleolar marker (D–F) and DAPI (blue) to counterstain nuclei (C–F). *NSun2* loss-of-function reporter lines (-/-) were obtained using *Nsun2*$^{tm1a(EUCOMM)Wtsi}$ mice carrying a GeneTrap in intron 6 of the *Nsun2* gene. For LacZ staining whole brains of *NSun2* +/- mice at postnatal day 3 were embedded in OCT. Sagittal frozen section were cut and stained using the recommended protocol (ftp://ftp.sanger.ac.uk/pub4/resources/mouse/sigtr/XGalStaining.pdf). Nuclear fast red (Sigma) was used as a counterstain according to the manufacturers' instructions. Quantification of NSUN2-positive nucleoli throughout the brain: For the quantifications, the relevant area of a wild-type brain was randomly selected and the percentage of NSUN2 nucleolar positive cells was counted. The corresponding section of the -/- brain was then selected and the same count was made. *N.B.* The Allen mouse brain atlas shows that there is significant expression of NSun2 in the hippocampus. However, we did not detect any appreciable nucleolar staining of NSun2 in the hippocampal regions although we did appreciate staining in areas that were also present in the -/- sections, and this therefore made it too difficult to assess hippocampal localization using our antibody (affinity purified, polyclonal Covalab). Although our antibody gave a degree of non-specific staining particularly in the cytoplasm, the nucleolar staining was highly specific.